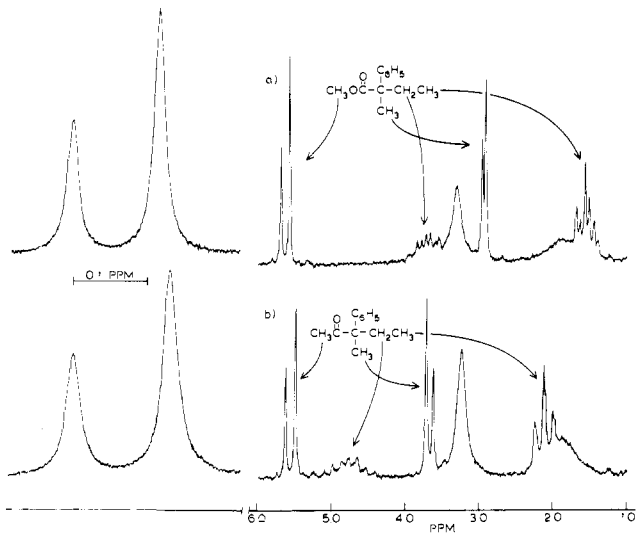


Figure 2. Spectra of CCl_4 solutions of 0.9 *M* **5** and 0.6 *M* **6** in the presence of 0.5 *M* **1**. Expanded spectra at left are for the downfield methyl resonances. Unassigned resonances are due to **1**.

the presence of tris(dipivalomethanato)europium(III) and in the presence of **1**. This comparison shows that similar pseudocontact shifts ($\Delta\delta$) are observed with the two reagents.[8] As illustrated by spectrum b, in the presence of **1** pseudocontact-shift differences for enantiomers are observed. The enantiotopic α-methyl singlets are separated 0.29 ppm and the β-methyl triplets are separated 0.22 ppm which corresponds to $\sim 2J$ and gives rise to a quintuplet. A more dramatic example of nonequivalence is illustrated by the lower spectrum in Figure 1. This spectrum of partly active 1,2-dimethyl-*exo*-2-norbornanol[9] in the presence of **1** shows large shift differences for corresponding methyl groups of the enantiomers. It is interesting to note that nonequivalence is largest (>0.5 ppm) for the least shifted methyl group (presumably the 1-methyl).

Effects of **1** on the nmr spectra of some other types of compounds are summarized in Table I. This table shows pseudocontact-shift differences ($\Delta\Delta\delta$) for the indicated enantiotopic protons. Nonequivalence was not observed with ethers. Magnitudes of pseudocontact shifts ($\Delta\delta$) and of $\Delta\Delta\delta$ depend on the ratio of **1** to substrate. Conditions have been optimized only for **4**. In this case $\Delta\delta$ and $\Delta\Delta\delta$ (for both methyl groups) increase with the **1**/**4** ratio until the latter reaches $\sim$0.7 after which there is no change. This suggests[8b] that at ratios >0.7 (optimum conditions) essentially all of the substrate is coordinated. In this connection it is significant that nonequivalence was not observed for protons that are enantiotopic by internal comparison,[4] *e.g.*, 2-propanol and dimethyl sulfoxide.

Nonequivalence of enantiomers is also observed with the praseodymium analog of **1** and shift differences are at least as large as with **1**; however, resolution is generally poorer. In this case induced shifts are in the upfield direction.[8c,9a]

The use of **1** for direct determination of enantiomeric compositions is illustrated by Figure 2 which shows spectra of optically active methyl 2-methyl-2-phenylbutanoate (**5**) and 3-methyl-3-phenyl-2-pentanone (**6**) in the presence of **1**. Both compounds were prepared from the same sample of partially resolved 2-methyl-2-phenylbutanoic acid (excess *S* isomer)[10] and have the same optical purities. For **5**, nonequivalence is observed for the *O*-methyl (*R*, 5.68 ppm; *S*, 5.56 ppm) and 2-methyl protons (*R*, 2.98 ppm; *S*, 2.93 ppm). Similarly, for **6**, nonequivalence is observed for the acyl-methyl (*R*, 5.62 ppm; *S*, 5.49 ppm) and 3-methyl protons (*S*, 3.72 ppm; *R*, 3.62 ppm). Expanded sweep widths of the downfield methyl resonances are shown to the left of the corresponding spectrum.[7] Peak areas of the expanded signals correspond to optical purities of 27.7% for **5** and 27.3% for **6** as compared to 25.8% for 5 and 25.4% for **6** determined from rotations.[10] It is noteworthy that in the spectrum of **6** the sense[2] of nonequivalence is reversed for the acyl-methyl and 3-methyl singlets. This indicates that nonequivalence results from intrinsicly different magnetic environments for coordinated enantiomers. Differences in stability constants for complexes of enantiomers may also contribute to nonequivalence.

Table I. Pseudocontact-Shift Differences for Enantiomers ($\Delta\Delta\delta$) in the Presence of **1**[a]

Compound	Proton	$\Delta\Delta\delta$, ppm
2-Octanol	α-CH_3	0.11
1,2-Dimethyl-*endo*-2-norbornanol	1-CH_3	0.37
	2-CH_3	0.33
1-Phenylethanol	α-H	0.30
1-Phenylethyl acetate	$-CO_2CCH_3$	0.18
1-Methyl-2-norbornanone	1-CH_3	0.17
3,3-Dimethyl-2-aminobutane	α-CH_3	0.28
cis-β-Methylstyrene oxide	β-CH_3	0.27

[a] Concentration of **1**, $\sim$0.4 *M* (200 mg/0.6 ml of CCl_4). Molar ratio of **1**/substrate, >0.6.

(8) For information regarding pseudocontact shifts and nmr shift reagents see (a) J. K. M. Sanders and D. H. Williams, *J. Amer. Chem. Soc.*, **93**, 641 (1971); (b) R. E. Rondeau and R. E. Sievers, *ibid.*, **93**, 1522 (1971); and (c) J. Briggs, G. H. Frost, F. A. Hart, G. P. Moss, and M. L. Staniforth, *Chem. Commun.*, 749 (1970), and references in these papers.

(9) H. L. Goering, C. Brown, S. Chang, and J. V. Clevenger, *J. Org. Chem.*, 34, 624 (1969).

(9a) NOTE ADDED IN PROOF. We have also investigated the europium and praseodymium chelates derived from 3-heptafluoropropylhydroxymethylene-*d*-camphor. These chiral chelates have nmr shift properties similar to the chelates derived from **3**. Nonequivalences for enantiomers are of about the same magnitude and resolution is similar for corresponding chelates.

(10) D. J. Cram and J. Allinger, *J. Amer. Chem. Soc.*, **76**, 4516 (1954); D. J. Cram, A. Langemann, J. Allinger, and K. P. Kopecky, *ibid.*, **81**, 5740 (1959).

(11) National Institutes of Health Predoctoral Fellow, 1968–1971.

Harlan L. Goering,* Jon N. Eikenberry, Gerald S. Koermer[11]
Department of Chemistry, University of Wisconsin
Madison, Wisconsin 53706
Received July 24, 1971

The Determination of Enantiomeric Purity Using Chiral Lanthanide Shift Reagents[1]

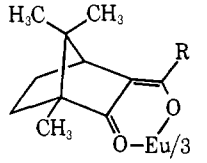
Sir:

Tris[*tert*-butylhydroxymethylene-*d*-camphorato]europium(III) (**1**) induces contact and/or pesudocontact shifts of different magnitudes in corresponding protons

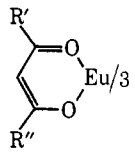
(1) Supported by the National Institutes of Health, Grant No. GM-16020.

of certain enantiomeric Lewis bases.[2,3] This observation demonstrates that nmr spectroscopy using chiral lanthanide chelates can in principle be employed to establish absolute enantiomeric purity; in practice, the utility of **1** is restricted to applications involving relatively basic and unhindered substrates (*e.g.*, primary and secondary amines), and fails with less basic substances. Here we wish to report the synthesis of the chiral shift reagents **2**–**7** and to outline data demonstrating the general applicability of these materials to the determination of the enantiomeric purity of relatively nonbasic substances.

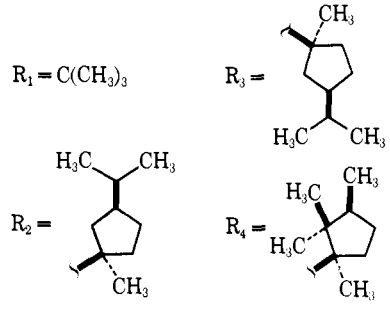
The β-diketone ligands from which these complexes are derived were prepared either by procedures analogous to that described previously for **1**[2] (for **2** and **3**), or by slow addition of the methyl ketone derived from R″ to a refluxing solution of the acid chloride derived from R′ in dimethoxyethane containing *ca.* 1 equiv of suspended sodium hydride and a catalytic amount of *tert*-butyl alcohol (for **4**–**7**).[4] Conversions of these



1, R = R_1
2, R = R_2
3, R = 77% R_3 + 23% R_2



4, R′ = R_2; R″ = R_2
5, R′ = R_2; R″ = R_4
6, R′ = 77% R_3 + 23% R_2; R″ = R_4
7, R′ = R_2; R″ = 77% R_3 + 23% R_2



β-diketones to the europium complexes **2**–**7** were accomplished as described previously;[2,5] crude complexes were purified by sublimation.

The *collective* utility of compounds **2**–**7** in separating the resonances of enantiomeric amines, alcohols, ketones, esters, and sulfoxides appears to be quite general; one or another of these shift reagents has induced shifts between enantiomers present in samples of the majority of these substances that we have examined. However, no single one of these reagents appears to be clearly superior to the others for every application.

(2) G. M. Whitesides and D. W. Lewis, *J. Amer. Chem. Soc.*, **92**, 6979 (1970).

(3) The utility of lanthanide chelates as shift reagents was first demonstrated by C. C. Hinckley, *ibid.*, **91**, 5160 (1969).

(4) Fenchoic acid was obtained from fenchone and campholic acid from camphor using standard procedures: *cf.* F. W. Semmler, *Chem. Ber.*, **39**, 2577 (1906); K. E. Hamlin and A. W. Weston, *Org. React.*, **9**, 1 (1957); F. E. L. Humbert, *Bull. Soc. Chim. Fr.*, 2867 (1966). The *d*-fenchone used in this study contained 23% of the *l* enantiomer. Carboxylic acids were converted to the corresponding methyl ketones and acid chlorides by standard procedures, using methyllithium and thionyl chloride, respectively.

(5) K. J. Eisentraut and R. E. Sievers, *J. Amer. Chem. Soc.*, **87**, 5254 (1965).

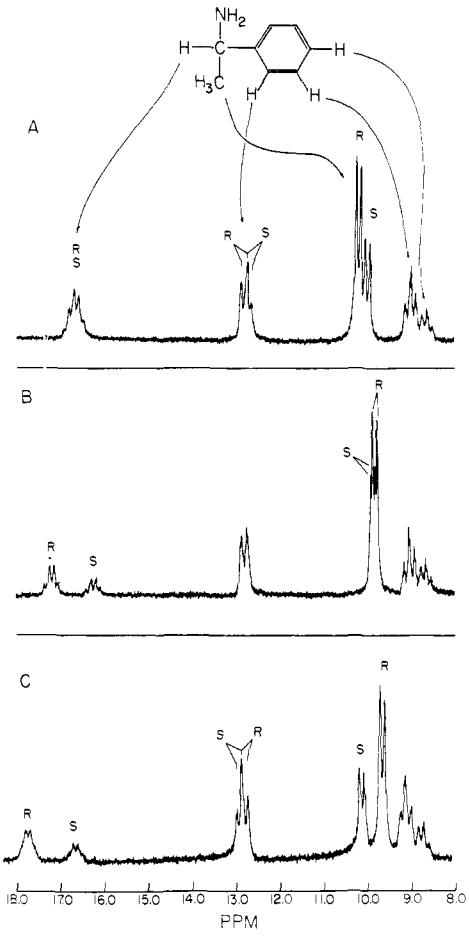
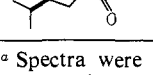


Figure 1. Spectra of solutions prepared from mixtures of (*R*)- and (*S*)-1-phenylethylamine in CCl_4 containing the chiral shift reagents **3** (0.20 *M*) (A), **2** (0.19 *M*) (B), and **7** (0.15 *M*) (C). In each solution, the *R* enantiomer is present in higher concentration than the *S*. Solutions are approximately 0.35 *M* in 1-phenylethylamine. The conditions under which these spectra were obtained are not necessarily those in which the enantiomeric shift differences are maximized.

Thus, representative data obtained for typical substrates with reagents **2** and **7** (Table I), and the full spectra shown in Figure 1, demonstrate an appreciable (and presently unpredictable) sensitivity of the shifts between resonances of enantiomers to changes in the structure of the shift reagent.

Table I. Differences in Resonance Fequencies (ΔΔδ, ppm) between Corresponding Protons of Enantiomers in the Presence of Shift Reagents **2** and **7**

Compound	ΔΔδ **2**[a]	ΔΔδ **7**[b]
$C_6H_5CH(CH_3)NH_2$	1.13	1.65
$C_6H_5CH(CH_3)NHCH_3$	0.28	0.46
$C_2H_5CHNH_2CH_3$	0.20	0.64
$CH_3CHNH_2CO_2C_2H_5$	0.75	0.97
$C_6H_5CCH_3(C_2H_5)OH$	0.32	0.25
$C_5H_{11}CHOHCH_3$	0.10	0.00
$C_6H_5CH_2SOCH_3$	0.32	0.40
$C_2H_5CH(O_2CCH_3)CH_3$	0.00	0.075
$C_2H_5CH(O_2CH)CH_3$	0.00	0.10
$CH_3COCH(CH_3)C_2H_5$	0.00	0.058
	0.00	0.050

[a] Spectra were obtained in CCl_4 solutions *ca.* 0.4 *M* in shift reagent and 0.3 *M* in substrate. [b] Solutions in CCl_4, *ca.* 0.25 *M* in shift reagent.

Two features of the presently available data suggest that the association and conformational equilibria responsible in part for the enantiomeric shift differences are complex. First, there is no apparent correlation between the magnitudes of the shifts induced in chemically distinct sets of enantiomeric protons and the structure of the shift reagents. Thus, the shift between the methyl protons of (*R*)- and (*S*)-1-phenylethylamine is small in solutions of **2** but moderately large in the presence of **3**, while the C*H* protons in the same samples show the opposite behavior (Figure 1). Second, the *sense* of the shift difference need not be the same for all the protons in enantiomeric substrates; each proton in (*R*)-1-phenylethylamine falls at lower field than the corresponding proton of the *S* enantiomer in solutions containing **1**,[2] while the protons of the methyl group of the *R* enantiomer resonate at *higher* field, and the C*H* of this enantiomer at *lower* field, than the analogous *S* protons, in the presence of **2** and **7**. Since the shielding experienced by protons in complexes of lanthanide shift reagents with substrates is a sensitive function of the geometry of these complexes,[6,7] and since the diastereomeric complexes formed as the result of coordination of enantiomeric bases to chiral chelates need not necessarily have closely related geometries, these observations are not surprising. However, they do suggest that *prediction* of the sense and relative magnitudes of shifts between enantiomers in these systems will be difficult,[8] and that in practical applications it may accordingly be worthwhile to examine several different chiral shift reagents to find empirically the one giving the most useful spectra.[8a]

Acknowledgment. We wish to thank Professor Harlan Goering for communicating results prior to publication[9] and for samples, and Hoffmann-LaRoche, Inc., for gifts of optically active compounds.

(6) B. L. Shapiro, J. R. Hlubucek, C. R. Sullivan, and L. F. Johnson, *J. Amer. Chem. Soc.*, **93**, 3281 (1971); P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *ibid.*, **92**, 5734, 5737 (1970), and references in each.

(7) For recent structural data on tris(β-diketo)lanthanide complexes, see J. C. A. Boeynes, *J. Chem. Phys.*, **54**, 75 (1971); W. D. Horrocks, Jr., J. P. Sipe III, and J. R. Luber, *J. Amer. Chem. Soc.*, **93**, 5258 (1971), and references in each.

(8) The prospects for utilizing chiral shift reagents to determine *absolute* configurations presently appear very dim. For successful applications of nmr spectroscopy in chiral solvents to determination of absolute configurations, see W. H. Pirkle, R. L. Muntz, and I. C. Paul, *ibid.*, **93**, 2817 (1971).

(8a) NOTE ADDED IN PROOF. Additional evidence supporting the hypothesis that the conformation of the β-diketone ligands around the europium ion is fairly flexible is provided by the observation by M. McCreary that it is possible to *induce* chirality in $Eu(DPM)_3$ by coordination with a chiral amine. Thus, a $\Delta\Delta\delta$ of 0.36 ppm is observed for the C*H*NH_2 resonance of racemic 1-phenylethylamine (0.45 *M*) in a $CDCl_3$ solution containing $Eu(DPM)_3$ (0.45 *M*) and (*R*)-*N*-methyl-1-phenylethylamine (0.64 *M*).

(9) H. L. Goering, J. N. Eikenberry, and G. S. Koermer, *ibid.*, **93**, 5913 (1971).

George M. Whitesides,* Daniel W. Lewis

Department of Chemistry, Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Received August 3, 1971

Experimental Evidence for the Assignment of α C*H* Peaks in the Nuclear Magnetic Resonance Spectra of Polypeptides

Sir:

In pmr spectra of polypeptides undergoing the helix–coil transition, the backbone resonances α C*H* and N*H* sometimes give rise to double rather than single peaks. Although these are usually assigned to largely coil and largely α helical conformations, two recent publications, one from Scheraga's laboratory[1] and the other from Tam and Klotz,[2] have proposed different assignments. Evidence is provided here to support the original assignment of the two peaks, first observed by Ferretti.[3]

If it is assumed that the helical and random coil conformations are both present in single polypeptide chains, the observation of double α C*H* peaks suggests that the helix–coil transition is a slow exchange phenomenon ($\tau \approx 10^{-2}$ sec).[4] This is in sharp contrast to the results of several relaxation experiments[5] on the transition that indicate fast exchange ($\tau \approx 10^{-7}$–10^{-8} sec).[5] Explanations of the double peak behavior based on slow exchange have been given by Bradbury and coworkers,[6] who propose protonation of the amide residues, and by Ferretti and coworkers,[7] who propose a nucleation step having a high potential energy barrier. Ullman[8] has shown, however, that it is not necessary to postulate slow exchange since polydispersity and end-of-chain effects are sufficient to give rise to multiple peaks even with rapid exchange. Experimental evidence has been presented by ourselves which supports the ideas of Ullman.[9] Recently, however, Scheraga and coworkers[1] and Tam and Klotz[2] have questioned the assignment of the double peaks. The former authors[1] assume that the resonance of α helical backbone protons will not be observable due to dipolar broadening and propose that the two peaks observed are due to acid-solvated and unsolvated coil residues. The latter authors,[2] on the basis of polyalanine spectra, reintroduce the suggestion of protonation by TFA and favor the proposals of Bradbury and coworkers[6] as to the origin of the double peak.

Scheraga's proposals may be simply tested as follows: if the resonance from helical residues is too broad to be observed then the α C*H* peak area observed in helix-supporting solvents represents only a small fraction of the total polymer and corresponds to the few residues remaining in an "unsolvated coil" state. Addition of strong acid causing a complete helix–coil transition should therefore result in a large increase in relative area of the peak at the chemical shift attributed to "solvated coil," since all residues will then contribute to the peak area. The same change in solvent composition should cause a much smaller effect on the areas of the side-chain peaks and Table I, therefore, gives the ratios of the total α C*H* area to that of

(1) F. J. Joubert, N. Lotan, and H. A. Scheraga, *Biochemistry*, **9**, 2197 (1970).

(2) J. W. O. Tam and I. M. Klotz, *J. Amer. Chem. Soc.*, **93**, 1313 (1971).

(3) J. A. Ferretti, *Chem. Commun.*, 1030 (1967).

(4) (a) J. L. Markley, D. H. Meadows, and O. Jardetzky, *J. Mol. Biol.*, **27**, 25 (1967); (b) E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Nature (London)*, **216**, 862 (1967); (c) J. A. Ferretti and L. Paolillo, *Biopolymers*, **7**, 155 (1969); (d) J. H. Bradbury and M. D. Fenn, *Aust. J. Chem.*, **22**, 357 (1969).

(5) (a) R. Lumry, R. Legare, and W. G. Miller, *Biopolymers*, **2**, 484 (1964); (b) T. K. Saksena, B. Michels, and R. Zana, *J. Chim. Phys. Physicochim. Biol.*, **65**, 597 (1968); (c) G. Schwarz and J. Seelig, *Biopolymers*, **6**, 1263 (1968).

(6) J. H. Bradbury, M. D. Fenn, and A. G. Moritz, *Aust. J. Chem.*, **22**, 2443 (1969).

(7) J. A. Ferretti, B. W. Ninham, and V. A. Parsegian, *Macromolecules*, **3**, 34 (1970).

(8) R. Ullman, *Biopolymers*, **9**, 471 (1970).

(9) E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Polymer*, **11**, 277 (1970).